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# Does an onion-enriched diet beneficially affect the microbiotal composition in healthy human subjects?

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## 1) Background:

Onions are rich in fructo-oligosaccharides (FOS), which are well acknowledged prebiotic substances. FOS consumption have previously been associated with an increased level of fermenting bacterial genera e.g. Lactobacillus and Bifidobacterium. Generally, these groups of bacteria are considered to have beneficial effects on the intestinal environment. The aim of the present study was to use quantitative PCR and DGGE to analyze the effects of onion consumption on the gut microbiotal profile.

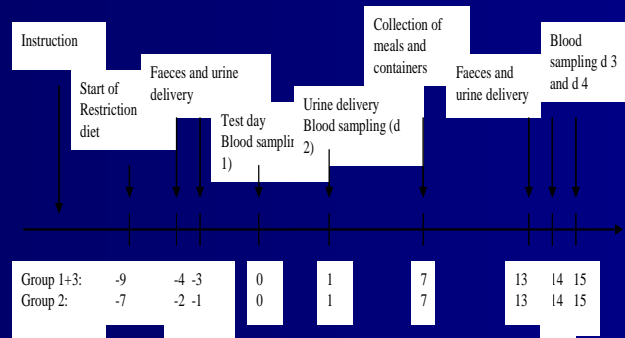
## 2) Study design:

- 10 human subjects (5 male + 5 females)
- 24-67 years old
- BMI: 25-35 kg/m<sup>2</sup>
- No other restriction diets allowed
- Randomized double-blind crossover design
- Two 14 day dietary intervention periods

- A) Onion enriched (Yellow/Red)
- B) Onion not enriched (Yellow/Red)

- 8 days restriction diet before each intervention period excluding onions and many other possibly confounding vegetables etc

## 3) Study timeline for one intervention period



## 4) qPCR results:

Total DNA was isolated from 4 stool samples (Sample Days 1+2, Before diet, and Sample Days 3+4, After diet) for each individual, for each of the two diets, using the Qiagen Stool Kit and subsequently quantitative real-time PCR was performed with **primers representing the Genera:**

**Lactobacillus, Bifidobacterium, Bacteroidetes, and Clostridium**

Note that no differences between Sample Days are present for any of the tested primers.

However, there are large individual differences in both the **Yellow** and **Red** diet groups.

t-test of differential expression in each individual showed no systematic effects (not shown)

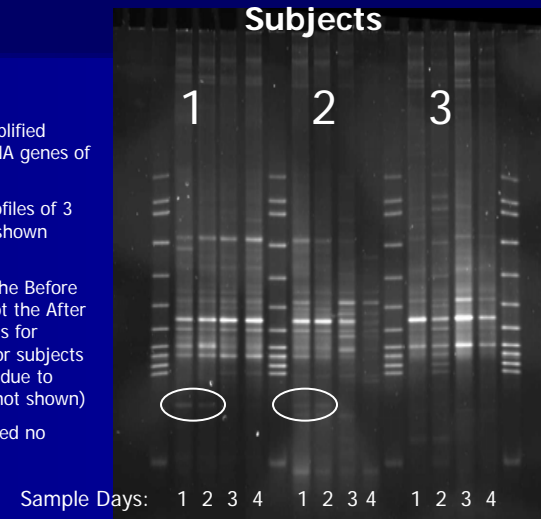
## 5) DGGE results:

DGGE was run on PCR amplified universal bacterial 16S rRNA genes of the isolated stool DNA.

Gel representing DGGE profiles of 3 subjects on **Yellow** diet is shown here.

Note the band present in the Before (sample days 1+2), but not the After (sample days 3+4) samples for individuals 1 and 2. Gels for subjects 4-6 needs to be run again due to insufficient PCR reaction (not shown)

Gels for the **Red** diet showed no obvious differences



	Yellow	Red
Strain/Test	2-way ANOVA Days (1) x Individuals (6)	2-way ANOVA Days (4) x Individuals (5)
Bifido	$F_{3,15}=0.197, p=0.95$ (no effect of Day) $F_{5,15}=3.73, p=0.022^*$ (clear Individual effect)	$F_{3,15}=1.11, p=0.38$ (no effect of Day) $F_{5,15}=5.15, p=0.0022^*$ (clear Individual effect)
Lactobacillus	$F_{3,15}=0.53, p=0.67$ (no effect of Day) $F_{5,15}=1.62, p=0.21$ (no Individual effect)	$F_{3,15}=1.56, p=0.24$ (no effect of Day) $F_{5,15}=0.32, p=0.90$ (no treatment effect)
Clostridia	$F_{3,15}=1.49, p=0.26$ (no effect of Day) $F_{5,15}=26.25, p<0.0001^*$ (clear Individual effect)	$F_{3,15}=1.62, p=0.23$ (no effect of Day) $F_{5,15}=22.46, p<0.0001^*$ (clear Individual effect)
Bacteroides	Failed – too many missing obs. $F_{1,21}=0.113, p=0.91$ (no treatment effect)	Failed – too many missing obs. $F_{1,21}=0.113, p=0.91$ (no treatment effect)

## 6) Conclusions:

- qPCR of selected primers showed no effect of treatment (**Red/Yellow**)

- Significant differences in individual Bifido, Lactobacillus, Clostridia and Bacteroides found

- DGGE of universal primers showed no or few obvious changes, but it would be a good idea to repeat the PCR, since gels representing half the test population were less efficient

- It is unfortunate that only 6 of 10 subjects finished the protocol. Makes it difficult to draw conclusions (or expect) findings

- The gel bands differing between Before and After in the DGGE gel merits some attention